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KURLOFF CELL LEVELS IN THE PERIPHERAL BLOOD OF NORMAL AND OESTROGEN TREATED GUINEA-PIGS

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Summary.—The range of normal total Kurloff cell levels in blood was 0–225 cells/mm³ in 150 male and 61–819 mm³ in 250 female guinea-pigs. Kurloff cell levels had a logarithmic frequency distribution in females and were positively skewed with a substantial number of zero values in males. A transient rise in Kurloff cell levels results after a single injection of aqueous oestrogen whereas a sustained increase in numbers of these cells occurs when oestrogen is administered in oily suspension.

THE KURLOFF CELL is a mononuclear cell possessing a characteristic proteoglycan containing inclusion body (Marshall and Swettenham, 1959; Dean and Muir, 1970) and is found in the blood and organs of the guinea-pig (Revell, Vernon-Roberts and Gray, 1971). The function of these cells is obscure. They are present in increased numbers in the blood and organs of guinea-pigs during pregnancy (Ledingham, 1940), and it has been suggested that Kurloff cells may play some part in protection of the trophoblast from immunological damage (Marshall *et al.*, 1971). Inhibition of macrophage migration by proteoglycan extracted from Kurloff cells has been demonstrated (Revell *et al.*, 1972), but there is no evidence at present that this material is released *in vivo*. Although it has been shown that the Kurloff cell may be coated with cytophilic antibody (Wilson and Coombs, 1971) and that a population of Kurloff cells form presumptive "T" cell rosettes (Revell, Wilson and Coombs, in preparation), the possible involvement of these cells in immunological processes is speculative. Cells of similar morphology have not been demonstrated in species other than the guinea-pig, but the presence of cells of similar function is indicated by the extraction and purification of proteoglycans chemically similar to Kurloff proteoglycan from the spleens of

sheep, pigs, rats and humans (Dean *et al.*, 1971). Human spleen proteoglycan also inhibits macrophage migration (Revell *et al.*, 1972).

In order to study the Kurloff cell *in vivo*, it is necessary to first establish the normal levels of this cell in the blood of the guinea-pig and thus provide a baseline for future experiments on factors affecting the Kurloff cell. The levels of Kurloff cells in the blood have been studied by various authors in the past and values expressed as the percentage of Kurloff cells in differential white cell counts (Nadel, 1952; Schermer, 1967; Sandberg, 1970) or the percentage of lymphocytes (Alexeieff and Joukoff, 1928; Semenskaja, 1930) or mononuclear cells (Smith, 1947; Bimes *et al.*, 1964) containing inclusion bodies. Babudieri (1938) provided information regarding the mean numbers of Kurloff cells/mm^3 of blood but only for small groups of animals. The total numbers of Kurloff cells/mm^3 of blood have not been studied in a large population of normal guinea-pigs.

Although various authors have reported an increase in Kurloff cells in the blood and organs of guinea-pigs following oestrogen treatment and during pregnancy (Semenskaja, 1930; Babudieri, 1938; Ledingham, 1940), these changes in blood levels have not been studied quantitatively in a way allowing statistical evaluation of results.

A quantitative assessment of the levels of Kurloff cells in the blood of normal guinea-pigs is given in the present paper, together with an evaluation of the effects of oestrogen administered in aqueous solution and oily suspension.

MATERIALS AND METHODS

Animals.—Normal male and female guinea-pigs of Dunkin Hartley strain weighing 350–500 g, belonging to a closed colony at the London Hospital were used throughout.

Total and differential white cell counts.—Between 0·3 and 0·5 ml of blood was obtained from guinea-pigs by cardiac puncture and placed in a tube containing ethylenediamineacetic acid (EDTA) (Sequestrine, 2·5 ml, Staynes Laboratories). Total white cell counts were performed in duplicate using an appropriately calibrated electronic white cell counter (Coulter Counter, Model A). Differential white cell counts were performed on air dried smears fixed in methyl alcohol and stained with Giemsa. The total number of Kurloff cells/mm^3 blood was calculated from the total and differential white cell counts.

Stimulation of Kurloff cell production by oestrogen treatment.—Stimulation of Kurloff cell production was carried out by 2 regimens: 8 normal female guinea-pigs were injected subcutaneously with a single dose of 100 mg of oestradiol-17B (Organon) dissolved in 0·5 ml of 2·5% ethyl alcohol; 12 normal females were injected subcutaneously with 100 mg of oestradiol benzoate (Organon) in a suspension in 0·2 ml of sterile arachis oil once weekly for 3 weeks; 12 control animals received 3 injections of 0·2 ml of arachis oil alone.

RESULTS

Levels of Kurloff cells in normal male and female guinea-pigs

Total and differential white cell counts were performed on the blood of 150 normal male and 250 normal non-pregnant female guinea-pigs in order to establish the normal Kurloff cell levels. The range of values was 0–225 Kurloff cells/mm^3 blood in males and 61–819 Kurloff cells/mm^3 in females. The arithmetic means were 55 and 261 Kurloff cells/mm^3 for males and females respectively. Both populations of values had a positively skewed frequency distribution (Fig. 1) and the arithmetic means are therefore misleading. The median values for males and females were 51 and 250 Kurloff cells/mm^3 respectively.

The distribution of the number of Kurloff cells in the blood of female animals

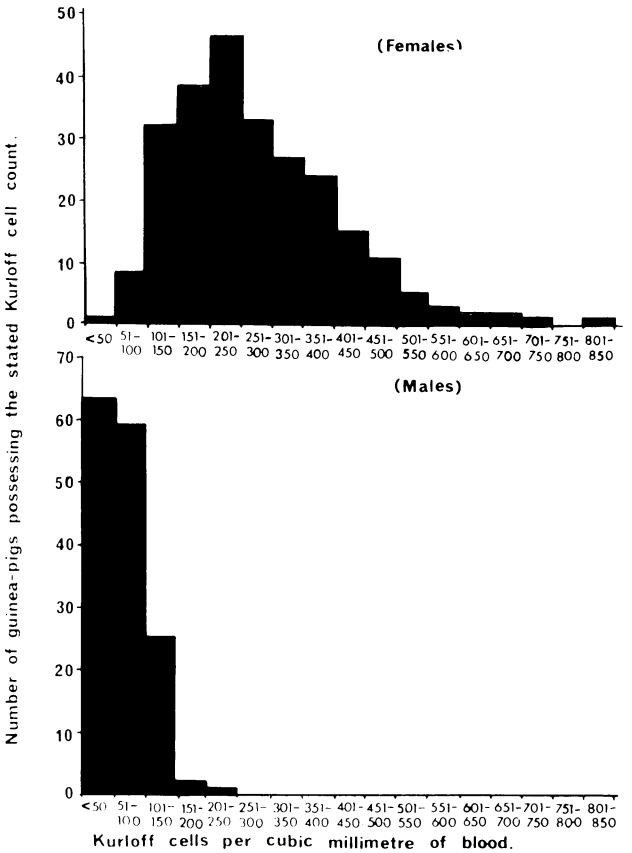

FIG. 1. Distribution of Kurloff cell levels in the peripheral blood of normal non-pregnant female and normal male guinea-pigs. There is a positively skewed distribution in both sexes and a large number of males have less than 50 Kurloff cells/mm³ blood.

was logarithmic, for when these values were expressed as the logarithm of the number of Kurloff cells/mm³ they assumed normal distribution (Fig. 2). The number of Kurloff cells in the blood of male animals was much lower and when 500 cells were counted in differential white cell counts no Kurloff cells were encountered in smears from 37 animals. These results, expressed as " nil " Kurloff cell counts, could represent the presence of a small number of cells, corresponding to less than 0·2% (1 in 500) of the differential white cell count, rather than a complete absence of Kurloff cells. An histogram of the logarithm of the Kurloff cells/mm³ blood of male guinea-pigs (Fig. 2) revealed that values above 50/mm³ assume a normal distribution leaving a single column of 37 animals in which no Kurloff cells were seen when 500 cells were counted.

Stimulation of Kurloff cell production by oestrogen treatment

Since it was shown that the values for Kurloff cell levels could be converted into a normal distribution by using the logarithm of the total Kurloff cell count, it became possible to establish confidence limits for levels in normal animals and

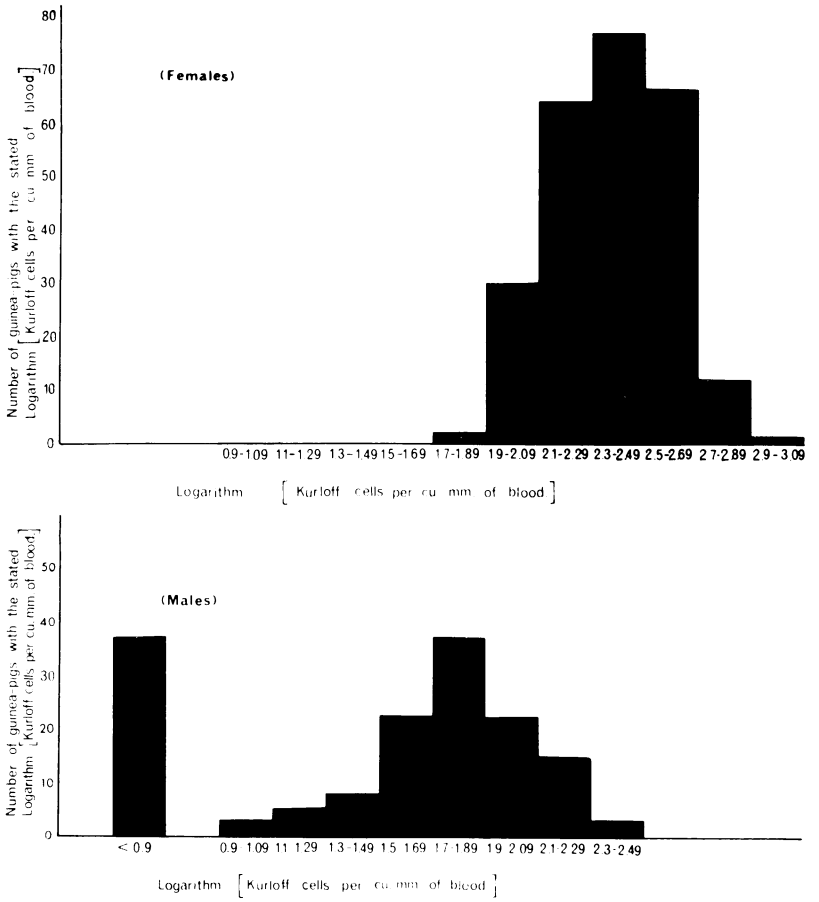

FIG. 2.—Distribution of the logarithm of Kurloff cells/mm^3 of blood. The values for females and most of the males assume a normal distribution. The remainder of the males represent 37 animals scored as " nil " Kurloff cells/mm^3.

investigate the effect of oestrogen administration on Kurloff cell production quantitatively. Female guinea-pigs were chosen in view of the difficulties experienced over counting small numbers of cells in normal male animals. Confidence limits for small groups of animals were calculated using the data obtained from the study of 250 normal female animals, and on the basis that 95% of values of a normal population fall within 2 standard errors of the mean.

The s.e. mean for a small population was calculated as follows:

$$\text{S.E. } (n) \qquad \frac{\text{S.D. } (N)}{n}$$

where S.E. (n) is the s.e. mean for the small experimental group.
S.D. (N) is the standard deviation of the logarithm of the Kurloff cells/mm^3 for N normal female guinea-pigs
and n is the number of animals in the small experimental group.

Ninety-five per cent of all normal groups of animals containing (n) animals lie within 2 s.e. (n) of the mean of the large population (N).

A group of 8 normal female guinea-pigs was given a single injection of oestradiol-17B in aqueous solution and the circulating Kurloff cell levels studied. Confidence limits were calculated for groups of 7 and 8 animals since one animal died during the experiment. On the second day after oestrogen administration, there was a transient rise in Kurloff cell levels which lasted for 24 h. This was significant

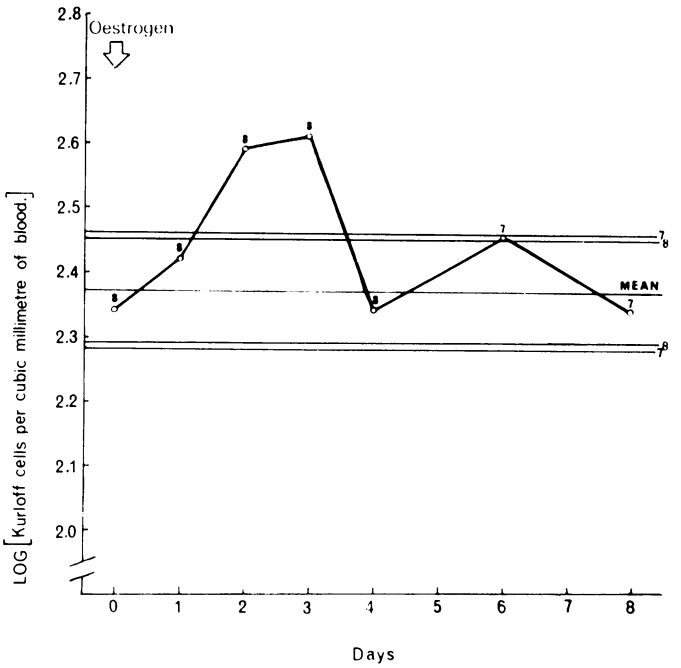

FIG. 3.—Number of Kurloff cells in peripheral blood of female guinea-pigs following a single injection of oestrogen in aqueous solution. Values are expressed logarithmically. There is an increase to levels outside the confidence limits ($P < 0{\cdot}05$) for normal animals occurring 2 and 3 days after oestrogen administration. Confidence limits for groups of 7 and 8 animals are shown (right). The number of animals for each group is shown for each point. One animal died during the experiment.

($P < 0{\cdot}05$) the mean value for the group being beyond the calculated confidence limit (Fig. 3).

The circulating Kurloff cell levels were measured in a group of 12 normal female guinea-pigs injected with oestradiol benzoate in oily suspension at weekly intervals and a control group of 12 animals treated with oil alone. Confidence limits were calculated for groups of 10, 11 and 12 animals since 2 guinea-pigs died in each group during the course of the experiment.

Four days after injection of 100 mg of oestradiol benzoate in arachis oil, the Kurloff cell level rose significantly above normal ($P < 0{\cdot}05$) (Fig. 4). It remained elevated during a further period of 2 weeks during which oestrogen was administered, returning to normal 19 days after the third and last injection. In contrast, the mean of 12 untreated normal animals remained within the confidence limits ($P < 0{\cdot}05$) throughout the period of the experiment (Fig. 4).

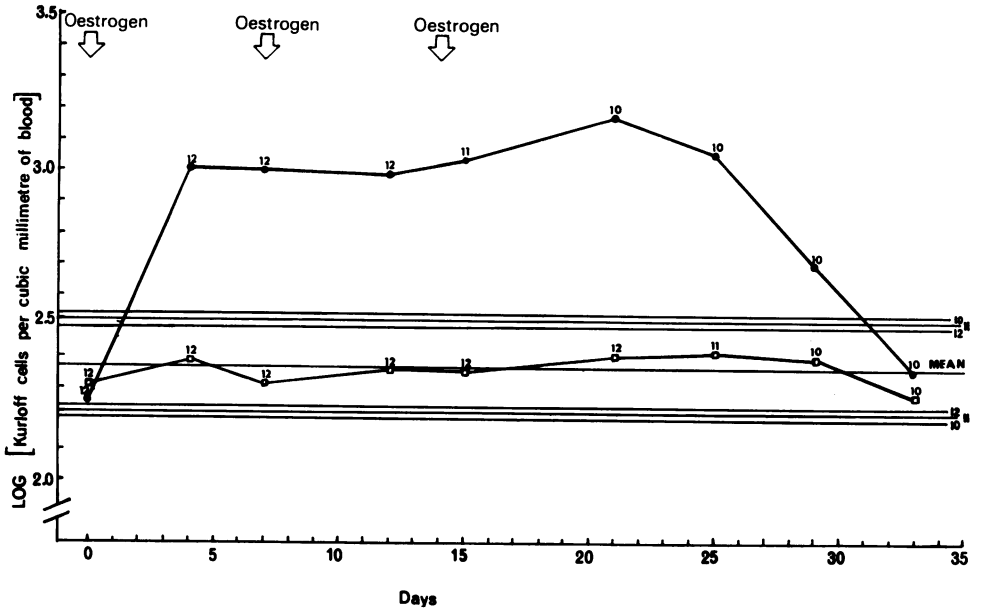

FIG. 4.—Number of Kurloff cells per/mm³ blood of female guinea-pigs expressed logarithmically. (●———●), during the course of 3 injections of oestrogen in oily suspension at 7-day intervals; (□———□), controls given 3 injections of oil alone. There is prolonged elevation of Kurloff cell levels during and following oestrogen treatment. Confidence limits are shown for groups of 10, 11 and 12 animals (right), and the number of animals in each group is shown by each point. Two animals died in each group.

These results showed that it is desirable to administer oestrogen in oily suspension at repeated intervals to obtain sustained elevation in Kurloff cell levels.

DISCUSSION

Normal blood levels of Kurloff cells

The total number of Kurloff cells in the peripheral blood of a large number of normal male and non-pregnant female guinea-pigs has been studied. Most previous authors have expressed Kurloff cell levels in terms of the percentage of these cells in differential white cell counts. However, Babudieri (1938) gave values for the total Kurloff cells per mm³ of blood but used only small numbers of normal animals. He also studied large numbers of normal animals but expressed these results as percentages of Kurloff cells in differential white cell counts. The shortcoming of using percentages alone is that this does not take account of the total white cell count.

In the present study, a clear difference has been demonstrated between the total Kurloff cell count in the peripheral blood of normal male and non-pregnant female guinea-pigs. Schermer (1967) stated that the Kurloff cell count in male guinea-pigs sometimes exceeds that of pregnant females, although he did not provide supporting evidence for this assertion. On the evidence of the effects of oestrogen stimulation demonstrated here, such an occurrence, if it ever happened, would be extremely rare.

The absolute numbers of Kurloff cells in the blood were found to have a positively skewed distribution in both male and female guinea-pigs. In the case of normal non-pregnant females, the distribution has been shown to be logarithmic. There are, however, 2 contributors to the skewness of the distribution of Kurloff cell numbers in the blood of normal male guinea-pigs. Most of the values assume a normal distribution when converted to logarithms, in much the same way as do those for females, while the remainder comprise a large group of " nil " values. The ability to detect occasional Kurloff cells in blood smears from male animals is limited when only 500 cells are counted. Ledingham (1940) commented that adult male guinea-pigs may possess fewer than one Kurloff cell/1000 white cells. It is not possible to state whether some normal male guinea-pigs do not possess Kurloff cells until differential counts of larger numbers of white cells have been carried out. The counting of a sufficient number of cells (possibly 10,000) to overcome this difficulty becomes impractical if groups of animals receiving different experimental treatments are to be examined on frequent occasions In contrast to male guinea-pigs, a minimum of 4 or 5 Kurloff cells was detected when 500 white cells were counted in smears of the blood of normal non-pregnant females. The use of females for quantitative studies of circulating Kurloff cell levels under different experimental conditions is therefore preferable.

The effects of oestrogen on Kurloff cell levels in the blood

A single dose of oestrogen in aqueous solution caused a significant but transient rise in Kurloff cell levels. The present results indicate that when oestrogen is administered in this way, daily injections may be required to bring about prolonged elevation of Kurloff cell levels. In this connection, soluble oestrogen was administered daily by Welsh (1966) to stimulate Kurloff cell production. In contrast, the Kurloff cell count in the blood remained elevated for 7 days after a single injection of oestrogen in oily suspension, and the increase in Kurloff cell count was easily sustained by further weekly injections of oestrogen in this form. To produce large numbers of Kurloff cells, previous workers have also used oily suspension (Ledingham, 1940; Marshall and Swettenham, 1959) or depot preparations (Nadel, 1952; Bimes *et al.*, 1964; Berendsen and Telford, 1967), but they did not assess the effect of these methods quantitatively or attempt to compare them with oestrogen in aqueous solution.

The difference between the effect of oestrogen in oily suspension and aqueous solution is almost certainly related to the difference in rate of their absorption from the site of injection. This is supported by the observation that the numbers of Kurloff cells in the blood did not return to normal limits until 19 days after the last injection of oestrogen in oily suspension. The time taken for Kurloff cell levels to reach a peak was less in the present study than the shortest period reported by Ledingham (1940), who obtained a peak in the percentage of Kurloff cells not sooner than 6 days after oestrogen administration.

In the light of the present findings, a regimen of weekly injections of an oily suspension of oestrogen is recommended for the sustained production of high levels of Kurloff cells in the blood. Since it is widely accepted that elevated Kurloff cell levels in the blood are accompanied by increased numbers of these cells in the organs, the same regimen of oestrogen treatment is also suitable for the study of Kurloff cells in the organs.

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